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BIOLOGICAL AND CHEMICAL CONTROL OF POTATO LATE BLIGHT DISEASE

BY

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ABSTRACT

Psudomonas fluorescens isolate PPf1 and *Bacillus* sp. isolate PB2 were selected among a collection of potato phyllosphere microorganisms as bio-agent against *P. infestans* under laboratory, greenhouse and field conditions. Obtained results revealed that, PPf1 and PB2 isolates were effective in reducing *P. infestans* mycelial growth. Bacterial cell suspension and/or their culture filtrate significantly inhibited the release of zoospores and cysts germination compared with the control ones. Selected bacterial isolates proved their ability to produce bio-surfactant and salicylic acid (SA) in their culture media. In addition, they effectively controlled potato late blight on detached and intact leaves under greenhouse conditions 2 days after spray application.

Acrobat fungicide strongly inhibited the mycelial growth, cysts germination of *P. infestans* than Previcur-N, while the opposite results were obtained in case of zoospores release. Both of fungicides tested controlled potato late blight disease under greenhouse conditions 2 days after foliage spray application. Acrobat showed a systemic activity against the disease as a foliar spray application than previcur-N. Moreover, the two fungicides were effective systemically as soil application. In field experiments, tested fungicides were effective than bio-control agents, which were more effective than the untreated control. Potato plants treated with fungicides and bio-agents had high salicylic acid (SA) contents compared with the control. Using fungicides and bacterial bio-control agents increased potato tuber yields, compared with the untreated control. The potato tuber yields were decreased by increasing the disease severity. Further studies are needed to improve the bio-control stability of bacterial isolates and increase their activity against potato late blight disease.

Key words: late blight, potato, *Phytophthora infestans*, bio-control, agents, *Psudomonas fluorescens*, *Bacillus* spp. salicylic acid, fungicides.

INTRODUCTION

Potato (*Solanum tuberosum* L.) late blight caused by *Phytophthora infestans* Mont. de Bary is one of the most important foliar and tuber diseases worldwide and the major yield-reducing factor of potato (Abu-El Samen *et al.* 2003 and Andrade-Piedra, *et al.* 2005). Nowadays there are lot of obstructions in using fungicides to control plant diseases, which are expensive, for their environmental hazards and since pathogen can develop resistance races to

fungicides (Visker *et al.*, 2003). On the other hand, biological control against fungal diseases of plants is eco-friendly and is a potential component of integrated disease management (IDM) as reported by Kishore, *et al.* (2005).

So, several investigators used the phylloplanic or rhizoplanic microorganism flora as bio-agents against phyto-pathogenic fungi or bacteria (Jindal *et al.*, 1988, Van Loon, *et al.*, 1997; Buchenaur 1998 & Kishore, *et al.* 2005). Other research workers, *i.e.* Eliseeva *et al.* (1995) and Filippov & Kuznetsova (1995) used *Pseudomanas* spp. and *Bacillus subtilis* to control the same pathogen on potato and or tomato. Moreover, plant growth promoting rhizobacteria (PGPR) are able to induce systemic resistance (ISR) in plants to control root and foliar diseases of several cultivated plants (Buchenauer, 1998; Chen *et al.*, 1999; Wie *et al.*, 1996; Enebak & Carey, 2000 & Kishore, *et al.* 2005).

On the other hand, the use of chemicals as fungicides proved to be the most effective methods to control several diseases (Stanghellini and Miller 1997). Field growing potato and/or tomato plants sprayed with fungicides one time and/or twice per week, reduced the area under disease progress curve (AUDPC), decreased yield losses compared with the results of fewer or non sprayed plants (Reiter, *et at.* 1995). Acrobat (dimethomorph 60 WP {DMM}) controlled *P. infestans* of potato and increased yield (Stuogiene 1997). It is effective against either metalaxyl-sensitive or metlaxyl-resistant isolates of *P. infestans* (Cohen *et al.*, 1995). Acrobat strongly inhibit *P. infestans* mycelial growth, zoospore encystment, cystospore germination *in vitro* (Grayson *et al.* 1996) and affect the oospores formation (Bissort *et al.*, 1997). Also, previcur-N (propamocarb-HCl) used to control tomato and potato late blight disease under laboratory, greenhouses and commercial field conditions. Which proved to be more effective as foliage than soil treatments, resulted in drastic loss of activity (Reiter, *et at.* 1995 and Klinkenberg *et al.*, 1998).

Therefore, the aim of the present study is to test selected bacterial isolates from a large collection of potato phyllosphere in addition to investigate their antagonistic effect against *P. infestans*, in laboratory, under greenhouse and field conditions in comparison with chemical fungicides. The role of biosurfactant (rhamnolipids) and salysilic acid production in bacterial culture media, as well as SA production in treated potato leaves, were investigated.

MATERIALS AND METHODS

1- Isolation, and inoculum preparation of *P. infestans:*

Phytophthora infestans was isolated from potato (leaves and stems) plants exhibited typical late blight symptoms collected from different locations in EL-Ismailia and El-Sharkia governorates. The selective method described by Sato *et al.* (1991) and the direct method described by Oyarzum *et al.* (1998) were used for isolating the late blight causal organism from collected samples.

The isolated *P. infestans* was identified according to the description of Ingram & Williams (1991) and Erwin & Ribeiro (1996) then cultivated on rye dextrose agar medium at 18 ± 2°C in the dark (Ribeiro, 1978). Sporangial suspensions were prepared from 14 days old cultures, then the suspension was

cooled at 4°C for 3-4 h to induce formation and release of zoospores. The suspension was diluted to 8 x 10^4 zoospores ml^{-1}. Each full true leaflets of potato plants were inoculated with four droplets (each 10µl) of the previously prepared zoospores suspension.

2. Isolation of different microorganisms from potato phyllosphere:

Healthy potato leaflets were collected from infected potato fields in winter cultivation. One gram of leaflets was transferred to Erlenmeyer-flask (250 ml) containing 99 ml of sterile distilled water (approximately 10^{-2} dilution). Flasks were shaken thoroughly on a mechanical shaker 150 rpm/minutes for 30 minutes. Serial dilutions up to 10^{-6} were prepared using sterile distilled water. One ml from 10^{-5} to 10^{-6} dilution was mixed with 9 ml nutrient agar and /or King's B medium (King *et al.*, 1954) in Petri dish, three plates were prepared for each dilution and incubated at 30 ± 2°C for 3 days to isolate the developing colonies. Bacteria were identified according to their shape, pigmentation and culture characteristics according to Buchanon *et al.* (1974).

3. *In vitro* experiments

3.1. Effect of the isolated bacteria and fungicide concentrations on mycelial growth of *P. infestans*:

The antagonistic interaction between the previously isolated bacteria on the mycelial growth of *P. infestans* was studied under laboratory conditions. Petri dishes (9 cm in diameter) containing rye agar medium amended with 3g yeast extract (Cohen, 1994) were inoculated in the center with a disk (9 mm in diameter) taken from 10 days old *P. infestans* cultures, then plates were inoculated with the selected isolated bacteria by streaking on the surface of the media beside the fungal growth (at the distance of 1.5 cm from the edge of the plates) with the aid of dual culture method.

The effect of different concentrations of the acrobat (0, 5, 10, 20 and 40 ppm) and prevecure-N (0, 500, 1000, 1500, and 2000 ppm) on the radial mycelial growth of *P. infestans* were examined. The aforementioned concentrations were added onto the sterile rye agar medium before solidification, then poured in Petri dishes. Each plate was inoculated with a growth disc (9 mm in diameter) of a 10 days old culture of *P. infestans*.

List of the fungicides tested

Trade name	Chemical structure	Active ingredient (a .i)	Recommended dose (ml/L)
Previcur-N®	Propamocab-HCl [propyl-N-(3-dimethylaminopropyl)-carbamate hydrochloride,	72,2 %	3
Acrobat (Dimethomorph)	Dimethomorph 13,9 % aqueous solution [(E,Z)- 4-(3-(4-chlorophenyl)-3-(3,4dimethyloxyphenyl) acryloyl) morpholine	14.7	2.5

P. infestans inoculated plates were used as control. Three replicates were used for each treatment, then plates were incubated at 18 ± 2°C. The diameter of the mycelial radical growth of different treatments was measured. When the plates of control were filled with the mycelial growth of *P. infestans*. The diameter of developed colonies were measured. Percentage of growth reduction were calculated from the following formula (Gang et al., 1994).

The percentage of growth reduction = (C-T/C) x 100.
Whereas T = mycelial growth in the treatment and C = mycelial growth in the control.

3.2. Effect of bacterial culture filtrates and fungicide concentrations on zoospores release and cysts germination of *P. infestans*:

The selected bacterial isolates were grown on King's B (King *et al.*, 1954) liquid medium for 7 days at 28 ± 2°C using shaking incubator. The resulted filtrates were obtained through Roth sparaten filter (25 mm).

Sporangial suspension of *P. infestans* ($8x10^4$) was mixed with an equal volume of each bacterial culture filtrate and or the aforementioned fungicide concentrations. The same volume of sterilized distilled water was added to the sporangial suspension in control treatment. Suspensions were incubated at 4°C for 3-4 hr, then the percentage of empty and normal sporangia were determined microscopically with the aid of the haemocytometer technique.

In other experiments zoospores were used instead of sporangia as previously and incubated at 18± 2°C for 16 h. Percentage of germinated and non-germinated cysts were determined microscopically with the aid of the haemocytometer technique.

3.3. Effect of bacterial suspension on zoospores release and cysts germination of *P. infestans*:

Obtained bacterial growth on King's B medium was blended for 1 min and the resulted suspensions containing bacterial cells and their filtrates in the liquid media were adjusted to be 10^8 CFU/ml.

The sporangial suspension of *P. infestans* ($2x10^4$) was mixed with an equal volume of each bacterial suspension separately, and with the same volume of King's B liquid medium of the control treatments, then the mixture was incubated at 4 °C for 3-4 h. The percentage of empty and normal sporangia were determined microscopically as mentioned before. The percentage of germinated and non-germinated cysts were determined microscopically using zoospores of *P. infestans* instead of sporangial suspension incubated at 18 18 ± 2°C for 16 h as mentioned before.

3.4. Free Salicylic acid production in bacterial culture medium:

Salicylic acid content in the culture filtrate was determined at 24-48 h by the methods descried by De Mayer & Höfte (1997). Culture filtrate was centrifuged at 14000 rpm for 10 min at 4°C, then one ml supernatant was mixed with 0.5 ml of 100% methanol and 50 µl trichloroacetic acid (TCA) 5 %. The volume was then adjusted to be 5 ml with de-ionized water and re-centrifuged at

14000 rpm for 10 min at 4 °C. Salicylic acid contents were analyzed by HPLC and calculated using the Kontron Data System 450-MT2/DAD.

3.5. Detection of the biosurfactant (rhamnolipids) produced by the bacteria:

A specific mineral salt (MS) medium as described by Siegmund and Wagner (1991) was used for this purpose. Selected bacterial isolates were inoculated at the surface of the MS medium, then incubated at 28± 2°C and checked daily. Developed clear zone around the bacterial growth, was measured.

4. *In vivo* experiments:

41. Effect of selected bacterial isolates on potato late blight disease incidence, under greenhouse conditions:

The most effective bacterial isolates (*P. fluorescens* isolate PPf1 and *Bacillus* sp. isolate PB1) on mycelial growth, release of zoospores and cysts germination of *P. infestans* were used to investigate their effects, against potato late blight disease, under controlled greenhouse conditions. The bacterial suspension prepared on King's B medium was blended and adjusted to contain 10^8 CFU/ml. Potato cv. Nicola plants, with 5-6 full leaf stage, in pots (20 cm in diameter) filled with sand/peat mixture (1:3 v/v) were sprayed with 50 ml/plant of each bacterial suspension and with King's B medium in the control ones. The sprayed plants were covered with plastic box and left under controlled greenhouse conditions (18 ± 2°C and 100 % RH).

Detached leaves previously sprayed with the PPF1 and PB1 bacterial cell suspensions mentioned above, were inoculated 2 days after treatment with 4 droplets (10µl) of *P. infestans* zoospores $8x10^4$ /ml as mentioned by Cohen (1994). Also the detached leaves were inoculated with 4 droplets (10µl) containing the mixture of the bacterial cell and fungal zoospores suspension (1:1 v/v) ast the same time (as a direct application). Intact leaves (all plants) were sprayed with 30 ml/plant of $8x10^4$ zoospores/ml of *P. infestans*.

Inoculated leaflets and/or plants were incubated at 18 ± 2°C and 100 % RH under plastic boxes in the greenhouse with a light and dark 16 and 8 h daily.

The disease incidence was evaluated after 5-7 days. Number and diameter of necrotic lesions (mm) as well as the blighted area/leaflet was determined (Cohen, 1994). The percentage of disease and percentage of protection was calculated (Cohen *et al.*, 1994) as follows:

percentage of protection = 100 x A/B

A= percentage of disease in treated plants.

B = percentage of disease in untreated plants (control).

(100 x blighted area in treated/blighted area in the untreated plants [control])

4.2. Effect of different fungicide concentrations on the late blight disease protection under greenhouse conditions:

Potato plants (5-6 true leaves old) were sprayed with different concentrations (1.25, 2.5 and 3.75 ml/l of acrobat and 1.5, 3 and 4.5 ml/l of previcur-N) on both upper and lower leaf surfaces. Control plants were sprayed with distilled water. The detached leaves and inoculation technique mentioned by

Cohen, 1994 were applied. The disease was evaluated after 5-7 days. Number and diameter of necrotic lesions (mm), the blighted area/leaflet, and the percentage of protection were calculated as mentioned in 4.1.

4.3. Systemic effect of tested fungicides against late blight disease severity, under greenhouse conditions:

4.3.1. Spray application:

Leaves no. 3 and 4 of 5-6 true leaves old potato cv. Nicola plants in pots, each containing one plant, were sprayed with the recommended rate of acrobat (2.5ml/l) and Prevecure-N (3 ml/l). The local effect was determined on the sprayed leaves (no 2 and 3), while the systemic effect was tested on the first upper unsprayed leaf (leaf no. 5), two days after spray application on potato leaves no. 3 and 4. Detached leaves assay was carried out, inoculation, incubation, percentage of disease and percentage of protection were calculated as mentioned before.

4.3.2. Soil drench application.

Fifty ml of the recommended rate of acrobat (2.5 ml/l) and previcur-N (3 ml/l) were added to the soil in pots (20 cm in diameter) filled with sand/peat mixture (1:3 v/v). Each pot containing one potato plant (5-6 completely developed true leaves old). Leaves no. 2, 3 and 4 were detached 2 days after soil application and inoculated with zoospore suspension of *P. infestans*. The results were calculated as mentioned before.

5. Field experiments

5.1. Effect of selected bacterial isolates and fungicides on potato late blight disease prameters under field conditions:

Bacterial isolates (*P. fluorescens* isolate PPf1 and *Bacillus* sp. isolate PB1) and tested fungicides were used to investigate their effect, as controlling agents against potato late blight disease in potato winter cultivation. These experiments were carried out at two successive growing seasons, in El-Tal El-Kabeer, Ismailia governorate, with 4 x 5 m experimental plots. Four replicates were used in complete random block design.

Field growing potato cv. Nicola plants (55-60 day old plants) were sprayed with each bacterial suspension 10^8 CFU/ml. once a week and repeated 6 times. Fungicides were applied as spray application once a two weeks and repeated 4 times. Four replicates were used in complete random block design. Quantification of disease incidence and severity at 7 days intervals starting at the appearance of late blight symptoms was determined using the 1-10 rating scale, started from 0 to 100 % infected leaves as mentioned by Vox (1993) and Cohen *et al.* (995). After harvest, the yield in each treatment was calculated per Feddan.

5.2. Free Salicylic acid (SA) determination in treated potato leaves under field conditions:

Free-SA was extracted from potato leaves according to the method of Malamy *et al.*, (1992). Samples of bacterial and fungicidal treated potato leaves under field condition were collected from the same levels, ground with liquid

nitrogen in 8 ml of 90% methanol using a pre-chilled mortar and pestle. The extract was centrifuged at 14000 rpm in Sorval ® SM 24 rotor for 15 min at 4°C. The pellet was re-suspended in 4 ml of 90% methanol and re-extracted at 14000 rpm for 15 min. Supernatants from both extractions were combined and dried under vacuum (150–170 m bar) at 45-50°C. The residue was re-suspended in 1 ml of 100% methanol and 50 µl trichloroacetic acid (5% TCA), the volume was then adjusted to 5 ml with de-ionized water and centrifuged at 14000 rpm for 10 min. The supernatant was applied to a HPLC equipped with Pharmacia LKB autosampler 2157 and Pharmacia LKB gradient pump 2249 (Germany) which was coupled to a GROM-SIL 120 O DS-3cp column (250 x 4 mm, 5 µm). Free-SA was detected by a fluorescence detector (LC304, Linear, Germany, excitation wavelength: 304 nm, emission wavelength 408 nm). Analysis of free-SA was carried out in methanol and sodium acetate buffer (20 mM Na-acetate buffer containing 10% methanol, pH 4.0). The concentrations of SA were calculated and evaluated using the Kontron Data System 450-MT2/DAD as described by Raskin *et al.* (1989) and Yalpani *et al.* (1991).

6. Statistical analysis:

Data obtained were subjected to statistical analysis proposed by Gomez and Gomez (1984), and means were compared using LSD multiple range test according to Duncan (1954).

RESULTS AND DISCUSSION

1. Biological control

1.1. *In vitro* experiments:

Pathogenic isolate of *Phytophthora infestans* was isolated from the collected samples. Several bacterial isolates (*Psudomonas fluorescens,* isolates and *Bacillus* spp. isolates) were isolated and identified among a collection isolated of potato phyllosphere.

Psudomonas fluorescens, isolate PPf1, was the most effective selected bacterial isolates in reducing radial growth of *P. infestans*, followed by *Bacillus* spp. isolate PB1 and PPf4, while PB3 followed by PB4 isolate were the least effective ones, the other tested isolates were in-between compared to the control treatment (Table 1).

Culture filtrates of PPf1 and PB1 bacterial isolates completely inhibited the release of zoospores and cysts germination of *P. infestans* compared with the control one. It was also clear that, cyst germination was more sensitive to culture filtrates than zoospores release (Table 2). The same results were obtained with the bacterial cell suspension of PPf1 and PB1 in their culture media on release of zoospores and cysts germination (Table 3).

Several bacterial genera had a good biocontrol activity against wide range of oomycetes fungi and other fungal genera (Eliseeva *et al.* 1995; Ahmed, *et al.* 2003 and Kishore *et al.*, 2005). The mode of action of the bacteria against mycelial growth, zoospores release and cysts germination might be due to

induction of anti-fungal compounds in its culture media (Niderman *et al.* 1995), production of laytic enzymes i.e. celluolytic, gliconolytic, chitinolytic, ß-1,3-gluconase as mentioned by Ng & Webster (1997) & Eash and El-Kohly (2005) or production of bio-surfactant which posses antifungal effects against germination of oomycetes cyst spores (Stanghellini and Miller, 1997).

Table (1): Antagonistic effect of different phylliospheric bacterial isolates against *Phytophthora infestans* measured as percentage of mycelial linear growth..

Bacterial isolates		Mycelial growth (%)	Mycelial growth reduction (%)
Control		100	0.00
*Pseudomonas fluorescens**	PPf1	33.35	66.65
	PPf2	65.33	34.67
	PPf3	74.67	25.33
	PPf4	62.00	38.00
LSD at 0.05		0.153	0.243
Bacillus sp*.	PB1	43.20	56.80
	PB2	71.95	28.05
	PB3	93.00	7.00
	PB4	78.67	21.33
LSD at 0.05		0.167	0.136

* bacterial isolates selected among a collection of bacterial isolates

Table (2): Effect of bacterial culture filtrates on the release of zoospores and cysts germination of *Phytophthora infestans* measured as percentage of empty sporangia

Bacterial isolates		Empty sporangia (%)	Empty sporangia reduction (%)	Germinated cysts (%)	Germinated cysts reduction (%)
Control		100.00	0.00	100.00	0.00
Pseudomonas fluorescens	PPf1	0.00	100.00	0.00	100.00
	PPf2	7.67	92.33	5.67	94.33
	PPf3	20.33	79.67	16.67	83.33
	PPf4	13.67	86.33	9.33	90.67
LSD at 0.5		0.189	0.180	0.182	0.160
Bacillus sp.	PB1	0.00	100.00	0.00	100.00
	PB2	22.50	77.50	12.50	87.50
	PB3	18.93	81.07	10.330	89.67
	PB4	15.20	84.80	13.67	86.33
LSD at 0.05		0.319	0.210	0.162	3.84

Table (3): Effect of selected bacterial cell suspensions on the release of zoospores and cysts germination of *Phytophthora infestans* measured as percentage of empty sporangia

Bacterial isolates		Empty sporangia (%)	Empty sporangia reduction (%)	Germinated cysts (%)	Germinated cysts reduction (%)
Control		100.00	0.00	100.00	0.00
Pseudomonas fluorescens	PPf1	0.00	100.00	0.00	100.00
	PPf2	6.33	93.67	4.33	95.67
	PPf3	9.67	90.33	8.33	91.67
	PPf4	7.33	92.67	3.67	96.33
LSD at 0.05		0.233	0.251	0.23	0.174
Bacillus sp.	PB1	0.00	100.00	0.00	100.00
	PB2	12.33	87.67	11.00	89.00
	PB3	19.67	80.33	17.33	82.67
	PB4	32.67	67.33	28.67	71.33
LSD at 0.05		0.212	0.0.210	0.167	0.183

The most effective bacterial isolates (PPf1 and PB1) were selected to their SA production in their culture medium which, found to be affected by incubation period. Bacterial isolate PPf1 produced high salicylic acid content than PB1 (Fig., 1). SA production by the bio-agent bacterial isolates was reported by several investigators (DeMayer and Höfte, 1997).

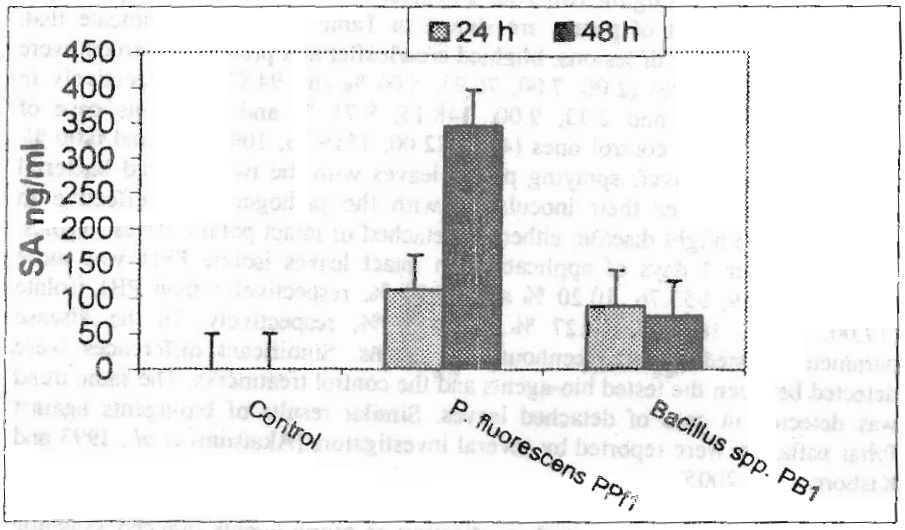

Fig. (1): Salicylic acid (SA) content produced by *Pseudomonas fluorescens* PPf1 and *Bacillus* spp. PB1 in liquid king's B medium after 24 and 48 h incubation periods.

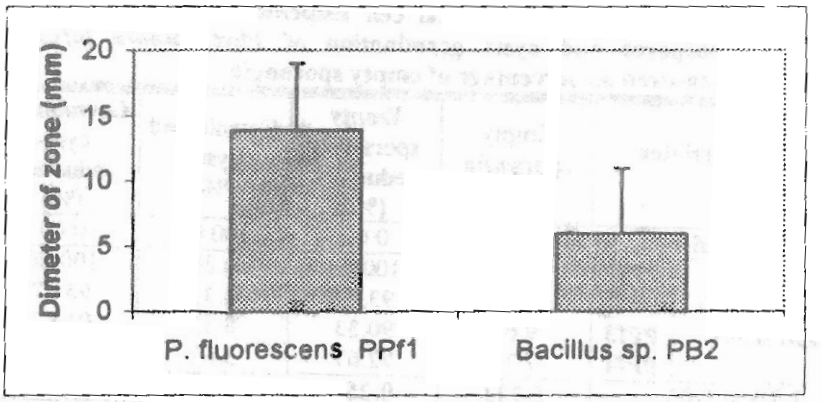

Fig. (2): Production of bio-surfactant by *Pseudomonas fluorescens* PPf1 and *Bacillus* spp. PB1 grown for 7 days on specific agar medium.

Production of bio-surfactant (Rahmenolipes) by the two selected bacterial isolates were tested on specific culture media. PPf1 isolate produced the highest amount of bio-surfactant than PB1 which had the wide clear zone around their colonies (Fig., 2). Similar results were detected by Stanghellini and Miller, 1997). Bio-surfactant, are known to act as antifungal against oomycets i.e. *Phytophthora* spp., *Pythium* spp. *Olipdium* spp. cysts germination (Stanghellini and Miller, 1997).

1.2. *In vivo* (greenhouse experiments):

Results of using the two selected bacterial isolates as a direct application to control late blight of potato are shown in Table (4). Results indicate that, number and diameter of lesions, blighted area/leaflet and protection percent were lower in treated leaves (2.00, 7.00, 76.93, 5.06 % and 94.93 % respectively in case of PPf1isolate and 2.33, 9.00, 148.15, 9.75 % and 90.25 ins case of PB1isolate) than the control ones (4.00, 22.00, 1519.76, 100.00 % and 0.00 %, respectively). Moreover, spraying potato leaves with the two selected bacterial isolates, 2 days after their inoculation with the pathogen were effective in controlling late blight disease, either on detached or intact potato leaves (Tables, 5 and 6). After 2 days of application on intact leaves isolate PPf1 was more effective (15, 9, 953.76, 10.20 % and 89.80 %, respectively) than PB1 isolate (17.00, 11.00, 1614.75, 17.27 % and 82.73%, respectively) in the disease parameters tested under greenhouse conditions. Significant differences were detected between the tested bio-agents and the control treatments. The same trend was detected in case of detached leaves. Similar results of bio-agents against foliar pathogen were reported by several investigators (Akatsumi *et al.*, 1993 and Kishore *et al.*, 2005).

In plant disease control, application of biotic agents induced systemic resistance (ISR) against fungi, bacteria and virus, through producing sidrophores, jasmonic acid (JA), ethylene and SA (Chen *et al.*, 1999), bacterial metabolites (Steiner and Schönbeck, 1995), induction of pathogeneses related (PR) proteins

such as PR-1, chitinase and ß,1-3,gluconase in the intracellular fluid of leaves (Chen *et al.*,1999) and or producing plant growth promoting substances (Chen *et al.*, 2000).

Table (4): Effect of two tested bacterial isolates on several parameters of potato late blight disease severity.

Bacterial isolate	Mean No. of lesions	Ø of lesion (mm)	Blighted area (mm^2)	Disease (%)	Protection (%)
Control	4.00	22.00	1519.76	100.00	0.00
Pseudomonas fluorescens PPf1	2.00	7.00	76.93	5.06	94.93
Bacillus sp. PB1	2.33	9.00	148.15	9.75	90.25
LSD at 0.05	0.323	0.762	1.680	0.103	0.120

Table (5): Effect of spraying two selected bacterial isolates two days after application on several parameters of potato late blight disease severity. Potato intact leaves were sprayed with *P. infestans* zoospore suspension

Bacterial isolates	Mean No. of lesions	Ø of lesion (mm)	Blighted area (mm^2)	Disease (%)	Protection (%)
Control	33.00	19.00	9351.70	100.00	0.00
Pseudomonas fluorescens PPf1	15.00	9.00	953.76	10.20	89.80
Bacillus sp. PB1	17.00	11.00	1614.75	17.27	82.73
LSD at 0.05	0773	2.77	1.27	0.769	0.543

Table (6): Effect of spraying selected bacterial isolates two days after application, on several parameters of potato late blight disease severity. Potato detached leaves were inoculated with 4 droplets of *P. infestans* zoospore suspension

Bacterial isolates	Mean No. of lesions	Ø of lesion (mm)	Blighted area (mm^2)	Disease (%)	Protection (%)
Control	4.00	23.00	1661.06	100.00	0.00
Pseudomonas fluorescens PPf1	2.33	11.00	221.32	13.32	86.85
Bacillus sp. PB1	2.67	13.00	354.22	21.32	78.68
LSD at 0.05	0.345	0.45	2.52	0.210	1.290

2. Fuungicides:

2.1. *In vitro* experiments

Different tested fungicide concentrations proved to be effective against the tested *P. infestans* isolate. Acrobat was highly effective on inhibiting the

mycelial growth, and cysts germination than previcur-N. However, the opposite results obtained in case of zoospores release (Table 7) were obtained. Acrobat at 5 ppm completely prevented the mycelial growth and cysts germination of tested *P. infestans* isolate. Similar results were obtained by Albert *et al.* (1988). The above mentioned data indicated that, the tested fungicides differed in their reaction against *P. infestans*. Difference in the reaction might be due to selective relationship between fungicides and fungus isolates (Cohen *et al.*, 1995; Reiter *et al.*, 1995 and Klinkenberg *et al.*, 1998). Kühn *et al.* (1991) found that, presence of Acrobat (dimeethomorph) in the media lead to loss of biochemical control processes involved in normal cell wall and induced changes in the mycelium ultra-structure of *P. infestans*. In addition, Albert *et al.* (1988) found that, the cyst spores of *P. infestans* lost its wall and become unable to germinate in presence of acrobat.

2.2. *In vivo* (greenhouse experiments):

In detached leaves, acrobat at a concentration of 3.75 ml/l was more effective in controlling potato late blight compared with the untreated control (Table, 8). Similar results with both fungicides were obtained by Cohen *et al.* (1995); Reiter *et al.* (1995); Bissort *et al.* (1997); Klinkenberg *et al.* (1998).

Data in table (9) indicate that, acrobat had a systemic effect against potato late blight disease when used as spray application (29.92 protection percent) while, previcur-N hadn't. Soil drench application revealed that, acrobat had the high systemic effect (100 protection percent) rather than previcur-N (85.22 protection percent) as shown in Table (10). These results are in agreement with the results obtained by Cohen *et al.* (1995).

Table (7): Effect of different concentrations of acrobat and previcur-N, on mycelial growth, zoospores release and cysts germination of *Phytophthora infestans*.

Fungicides Concentrations (ppm)		Mycelial growth (%)	Zoospores release (%)	Germinated cysts (%)
Control	0	100.00	100.00	100.00
Acrobat	5*	0.00	100.00	0.00
	20	0.00	93.33	0.00
Mean		0.00	96.67	0.00
LSD at 0.5		3.75	0.96	3.75
Prevcur-N	500	28.33	100.00	13.33
	1000	16.25	100.00	7.67
	1500	11.00	94.33	0.00
	2000	0.00	87.00	0.00
Mean		13.89	95.33	5.25
LSD at 0.5		1.75	1.30	3.56

Acrobat at 5 and 10 ppm showed the same results.

Table (8): Effect of different concentrations of two fungicides tested on several parameters of potato late blight disease severity, using detached leaves method.

Treatments	Mean no. of lesions	Ø of lesion (mm)	Mean blighted area (mm^2)	Disease (%)	Protection (%)
Acrobat					
Control	4.00	22.67	1613.74	100.00	0.00
1.25 ml/l	1.67	9.67	122.59	7.60	92.40
2.5 ml/l	0.00	0.00	0.00	0.00	100.00
3.75 ml/l	0.00	0.00	0.00	0.00	100.00
Mean	1.42	8.09	434.08	26.9	73.1
Previcur-N					
Control	4.00	22.67	1613.74	100.00	0.00
1.5 ml/l	2.33	11.00	221.32	13.71	86.29
3 ml/l	1.33	5.00	26.10	1.62	98.38
4.5 ml/l	0.00	0.00	0.00	0.00	100.00
Mean	1.92	9.67	465.21	28.83	71.17

LSD at 0.05

	No. of lesions	Ø of lesion	Blighted area	Protection (%)
Con.	0.798	0.393	4.19	3.75
Fungicides	0.267	0.189	2.25	1.82
Fungicides X Con.	0.745	0.834	5.50	4.96

Table (9): *Local* and systemic activity of two fungicides as foliar application, on several parameters of potato late blight disease severity, using detached leaves method.

Treatments	Treated leaves (L)			
	Mean no. of lesions	Ø of lesion (mm)	Mean blighted area (mm^2)	Protection (%)
Control	4.00	18.67	1094.51	0.00
Acrobat 2,5 ml/l	0.00	0.00	0.00	100.00
Pervicur-N 3 ml/l	1.67	5.00	32.77	97.01
	The upper untreated leaves (S)			
Control	4.00	16.33	837.34	0.00
Acrobat 2,5 ml/l	4.00	13.67	586.77	29.92
Pervicur-N 3 ml/l	4.00	16.33	837.34	0.00

LDS at 0.05

	No. of lesions	Ø of lesion	Blighted area	Protection (%)
Treatments (T)	1.31	0.59	7.99	5.90
Local (L) & Systemic (S)	0.56	0.23	2.98	1.99
T x L & S	1.92	0.89	11.36	7.67

Table (10): Systemic activity of two fungicides as soil application on several parameters of potato late blight disease severity, using detached leaves method.

Treatments	Mean No. of lesions	∅ of lesion (mm)	Mean blighted area (mm^2)	Disease (%)	Protection (%)
Control	4.00	21.67	1474.51	100.00	0.00
Acrobat 2,5 ml/l	0.00	0.00	0.00	0.00	100.00
Pervicur-N 3 ml/l	4.00	8.33	217.88	14.78	85.22
LSD at 0.5	Sig.	0.98	5.93	6.32	6.21

Sig. = The results were significant at 0. 5 %

3. Field experiments:

Under field conditions and in both growing seasons bacterial isolates tested affecting potato late blight disease, whenever, the disease severity was low or moderate, while the efficacy of disease control was decreased by increasing the disease severity at 89 days after sown Tables (11 and 12).

Controlling effects of selected bacterial isolates against potato late blight disease might be due to produce anti-fungal compounds, laytic enzymes in its culture media (Akatsumi *et al.* 1993). It might be also due to chitinase and ß-1,3-gluconase enzymes (Velazhahn *et al.*, 1999) production of promoting plant growth (Ng & Webster, 1997).

Spray application of acrobat (2.5 ml/l) was the most effective in controlling potato late blight under field conditions even at the highest potential of the disease in both growing seasons (Tables 11 and 12).

Application of acrobat, previcur-N and selected bacterial isolates resulted in an increase of potato tuber yield (Table, 13). Maximum potato tuber yield of 16.12 ton per feddan at the first season and 9.84 ton per feddan at the second one (146.49 and 151.16 % higher than control respectivily) was obtained by acrobat followed by Previcur-N (144.54 at the first) then PPf1 isolate (115.27 and 113.55 % higher than control respectively) and PB1 (104.50 and 110.53 % higher than control respectively) as shown in Table (13). Yield of the tested bacterial isolates at the second season was higher than the yield of previcur-N. In addition, potato tuber yield was low at the second season, whenever, the disease was sever. It clear that, there was a positive corelation between the disease and yield losses. Similar results were obtained by Kishore *et al.* (2005). Albert *et al.* (1988) found that, cinnamic acid derivative dimethomorph (Acrobat) possess translaminar activity and is systemic via root uptake, showed high activity against fungi from the genus *Phytophthora* and members of the Peronosporaceae. In addition, presence of acrobat in the media led to loss of biochemical control processes involved in normal cell wall and induced changes in the mycelium ultra-structure of *P. infestans* (Kühn *et al.* 1991).

Table (11): Effect of selected bio-agents bacteria and fungicides on potato late blight under field conditions at different times after sown during at 2001-2002 growing season.

Treatments	No. of diseased plants					
	Days after sown					
	75	84	91	98	105	M
Control	33.67	37.00	79.00	94.00	137.67	76.33
Pseudomonas fluorescens PPf1	3.00	3.33	49.33	49.33	100.33	41.3
Bacillus spp. PB1	0.67	1.00	4.67	8.67	81.67	19.33
Acrobat 2.5 ml/l	0.33	0.33	1.00	1.00	1.67	0.87
Pervicur-N 3 ml/l	0.67	1.33	2.67	3.33	3.67	2.33
LSD at 0.05 =Time		9.77				
Treatments		13.79				
Time X treatments		19.64				
	Disease severity					
Control	8.33	16.67	63.33	73.33	98.33	52
Pseudomonas fluorescens PPf1	1.67	1.67	3.67	20.03	71.67	19.74
Bacillus spp. PB1	0.00	0.00	1.70	1.70	58.33	12.35
Acrobat 2.5 ml/l	0.00	0.00	0.03	0.03	0.67	0.15
Pervicur-N 3 ml/l	0.00	0.00	0.03	0.03	1.70	0.35
LSD at 0.05 =Time		5.26				
Treatments		15.49				
Time X treatments		27.1				

Table (12): Effect of selected bio-agents bacteria and fungicides on potato late blight under field conditions at different times after sown during at 2002-2003 growing season.

Treatments	No. of diseased plants					
	Days after sown					
	75	84	91	98	105	M
Control	35.00	56.67	100.00	100.00	100.00	78.33
Pseudomonas fluorescens PPf1	20.00	43.00	96.67	100.00	100.00	71.93
Bacillus spp. PB1	16.67	30.00	80.00	100.00	100.00	65.33
Acrobat 2.5 ml/l	10.00	28.33	70.00	100.00	100.00	61.67
Pervicur-N 3 ml/l	0.00	0.00	8.33	21.67	83.33	22.67
LSD at 0.05 =Time		8.37				
Treatments		13.65				
Time X treatments		22.35				
	Disease severity					
Control	5.67	20.00	90.00	95.00	100.00	62.13
Pseudomonas fluorescens PPf1	2.00	9.00	65.00	88.33	100.00	52.87
Bacillus spp. PB1	1.33	3.33	42.00	60.00	96.67	40.67
Acrobat 2.5 ml/l	1.67	6.70	36.67	65.00	75.00	37.01
Pervicur-N 3 ml/l	0.00	0.00	0.01	0.07	25.33	5.08
LSD at 0.05 =Time		8.90				
Treatments		12.85				
Time X treatments		23.25				

Table (13): Effect of bio-agents selected and fungicides on potato yield (ton/feddan) under field conditions at two growing seasons 2001-2002 and 2002-2003.

Treatments	Growing season			
	2001-2002		2002-2003	
	Yield (ton)/feddan	Production (%)	Yield (ton)/feddan	Production (%)
Control	11.01	100.00	6.51	100.00
P. fluorescens PPf1	12.68	115.27	7.39	113.55
Bacillus spp. PB1	11.50	104.50	7.18	110.53
Acrobat 2,5 ml/l	16.12	146.49	9.84	151.18
Pervicur-N 3 ml/l	15.91	144.54	6.99	107.26
LSD at 0.5	0.62	0.93	0.35	1.32

Salicylic acid (SA) contents of potato treated plant leaves were significantly high in treated leaves than in similar leaves of untreated control. Treatment with acrobat produced the highest level (9.43 and 8.86 at both season tested respectively ng/g fresh weight) followed by Prevecur–N, while PB1 was the least one (Fig., 3). Salicylic acid may plays an essential role in induction of local acquired resistance (LAR) and systemic acquired resistance (Sathiyabama & Balasubramanian, 1998). Salicylic acid also serves as endogenous signal required for induction of SAR (Raskin *et al.*, 1989; Yalpani *et al.*, 1991; Coquoz *et al.*, 1995).

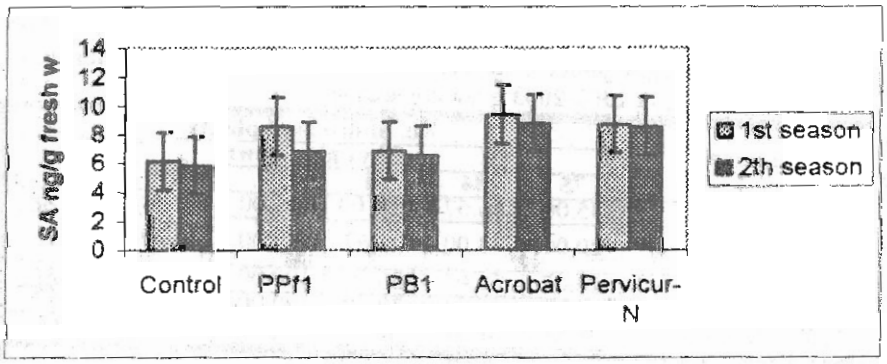

Fig. (3): Effect of bio-agents selected and fungicides on free SA contents of potato leaves under field conditions at two growing seasons 2001-2002 and 2002-2003. LSD at the first season was 0.512 and 0.198 at second one.

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المقاومة الحيوية والكيماوية لمرض اللفحة المتاخرة علي البطاطس

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تم دراسة الفعل المثبط تحت ظروف المعمل لعزلات بكتيرية تم عزلها وانتخابها من سطح أوراق البطاطس. وأوضحت النتائج المتحصل عليها أن العزلة PPf1 من *Psudomonas fluorescens* يليها عزلة PB2 التابعة لجنس *Bacillus* كانتا أكثر فعالية في تثبيط النمو الخطي وتحرر الجراثيم السابحة وكذا إنبات الجراثيم المتحوصلة للفطر فيتوفثورا انفستانس المسبب لمرض اللفحة المتأخرة في البطاطس. كما كان لمعلق خلايا البكتريا المختبرة نفس الأثر المثبط علي تحرر الجراثيم السابحة وإنبات الجراثيم المتحوصلة للفطر. أوضحت النتائج أيضا قدرة عزلتي البكتريا تحت الاختبار علي إنتاجها لحامض السالسيلك والرامنوليبيد في بيئة النمو. وقد كان لكلا العزلتين المقدرة علي مقاومة مرض اللفحة علي الأوراق المنزوعة وعلي النباتات الكاملة بعد يومين من المعاملة، وذلك تحت ظروف الصوبة.

أظهر المبيد الفطري أكروبات فعالية عالية في تثبيط النمو الخطي وكذا إنبات الجراثيم المتحوصلة للفطر فيتوفثورا انفستانس أكثر من المبيد بريفيكيور-ن بينما كان الأخير أكثر فعالية في تثبيط وتحرر الجراثيم السابحة. قد أدت المعاملة رشا بتركيزات

مختلفة من كلا المبيدين إلي مقاومة معنوية للمرض علي الأوراق المنزوعة بعد يومين من المعاملة في حين أظهر مبيد أكروبات نشاطا جهازيا ضد المرض عند استعماله رشا علي الأوراق وعلي العكس فإن بريفيكيور-ن لم يكن له نفس التأثير، بينما أظهر كلا المبيدين تأثيرا جهازيا ضد المرض عند إضافتهما للتربة.

أظهرت المبيدات الفطرية فعالية أكثر من كائنات المقاومة الحيوية في مقاومة المرض تحت ظروف الحقل في كلا موسمي الاختبار. حيث اثبت مبيد أكروبات فعالية أكثر تلاه بريفيكيور-ن ثم المعاملة بالبكتريا، التي كان لها نشاطا واضحا في مقاومة المرض عند مستوي الإصابة الضعيفة والمتوسطة وانخفضت فعاليتها بزيادة شدة المرض، وقد كان هذا التأثير واضحا في نهاية موسم النمو. وأدت المعاملة بالمبيدات الفطرية والبكتريا المعزولة من سطح أوراق البطاطس إلي زيادة إنتاجية محصول درنات البطاطس مقارنة بالنباتات غير المعاملة. وقد كان أعلي محصول عند استخدام مبيد أكروبات في كلا الموسمين تلاه مبيد بريفيكيور-ن في الموسم الأول ثم المعاملة بالبكتريا. وفي الموسم الثاني كان محصول المعاملة بالبكتريا أعلي من محصول المعاملة بالمبيد بريفيكيور-ن. وقد وجدت علاقة طر دية بين شدة المرض والخسارة في المحصول. أظهرت النتائج زيادة في محتوي أوراق البطاطس المعاملة بأي من المبيدين أوالبكتريا المعزولة من سطح أوراق البطاطس تحت الاختبار زيادة في محتوي الأوراق من حمض السالسيلك، مقارنة بالنباتات غير المعاملة. إضافة لما سبق،تحتاج هذا الدراسة لمزيد من البحث خصوصا لتحسين ثبات البكتريا علي سطح الأوراق وزيادة فعاليتها في المقاومة الحيوية للمرض مما يحسن من أداءها في المقاومة.